sageHLS

High Molecular Weight DNA Library System

Sage Science Inc. Suite 2400 500 Cummings Center Beverly, MA. 01915

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Indicates disposal instruction.

DO NOT throw this unit into a municipal trash bin when this unit has reached the end of its lifetime. To ensure utmost protection of the global environment and minimize pollution, please recycle this unit.

SageHLS Operations Manual Revision Change Log

Last	New			
Rev	Rev	Date	Page#	Notes
С	D	12/12/17	Appendices, Sections 6.16 – 6.24	Workflow guides added as appendices. Software Log Review and software updating added.
D	ш	2/8/18	Section 13 HIT kits	Several workflow changes see Section 13 change log
E	F	6/18/18	Section 13 HIT kits	Several workflow changes see Section 13 change log
E	ш	6/18/18	Section 12 HEX kits	Updated NEB Fragmentase ordering part number. Revised Workflow File names.
F	G	8/17/18	Section 12 and 13	Update cassette buffer filling instructions
F	G	8/17/18	Section 12	Corrected Dilution and math are for E.coli fragmentase dilution.
G	Н	10/24/18	Section 13	Remove -20°C storage recommendation for cas9 gRNA assemblies (not verified)
Н	Ι	3/12/19	Section 13	Added beta cyclodextrin to HIT kits to reduce SDS in qPCR assays
	J	12/12/19	All sections Over 6	Remove and separate all workflow guides.
J	K	6/1/21	ii and 1-2	Added WEEE instrument recycling disposal statement

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EQUIPMENT RATING AND SAFETY

1.1 Safety Icons Used in this Manual

In this manual, the following icons will be used to provide the user with information pertinent to the use of the SageHLS.



Warning! Warns the user that injury or instrument damage may occur if the contents of the warning are not properly followed.



High Voltage! Warns of the risk of electrical shock if the contents of the warning are not properly followed.



Hot Surface! Warns of a hot surface that may cause injury or irritation if touched.



Important! Provide important information about the proper use of the system that may influence the quality of the result.



Information. Provides additional information regarding the function of the system or applications for which is used.

1.2 SageHLS Equipment Ratings

- Input supply voltage: 100-240V
- Frequency range: 50-60Hz
- Current Rating: 2.8 A

1.3 SageHLS Instrument Safe Use Guidelines

The SageHLS system is designed to operate under the following environmental conditions:

- Pollution Degree 2
- Installation category 2
- Altitude 2000m
- Indoor use
- Ambient temperature 17-25°C
- Humidity 10-80%, non-condensing



Warning! The SageHLS was designed to be operated on a flat surface. Do not operate on a tilted surface or tilt during operation.

Standard laboratory precautions should be taken when handling Pippin Prep Gel cassettes and operating the Pippin Prep:

- Wear a lab coat, safety glasses, and gloves.
- Use in proximity of an eye wash station and/or running water.

Disposal



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1.4 Consumables Safe Use Guidelines

There are two types of consumables that are used on the SageHLS instrument:

- 1. **Agarose gel cassette.** This is an item that is manufactured by Sage Science. It consists of a styrene cassette into which an agarose gel is cast. The gel is immersed in liquid electrophoresis buffer, and all ports and openings are sealed with adhesive tape. The buffer is formulated with Tris-TAPS and is not hazardous.
- 2. **Reagent Kits**. Reagent kits consist of reagent formulations that are manufactured by Sage Science or third-party suppliers that are provided as liquids in tubes or bottles. These include lysis reagents, buffers, enzymes (dilute, in buffer), dyes, and in some cases short DNA or RNA that have been manufactured (not originating from plants or animals). All components are non-hazardous.

A partial list of reagent concentrations is provided on the next page.



Important! Reagent kits do <u>not</u> contain hazardous, known mutagenic, or known carcinogenic substances. Some chemicals may be skin irritants at higher concentrations than supplied in our kits (e.g. Sodium Dodecyl Sulfate).



Important! Users should refer to the Material Safety Data Sheets (MSDS) for comprehensive outline of the consumables safety classifications. These are posted at www.SageScience.com/product-support/sagehls-support/



Important! Users should use the SageHLS and related consumables in accordance to safe laboratory guidelines including the use of gloves, safety glasses, and lab coats.



Important! SageHLS consumables do <u>not</u> contain any gases or volatile compounds that can adversely affect health.

Unpacking and Installation

2.1 Unpacking the SageHLS

The SageHLS instrumentation is shipped in two boxes: one will contain the SageHLS and Accessories and the second box will contain the computer monitor in the manufacturer's original packaging. With the boxes in the upright position, open and confirm that the following items are enclosed:

Monitor

- LCD computer monitor
- Video cable
- Power cord

SageHLS

- SageHLS Instrument
- Accessory box
 - Computer keyboard, USB
 - Computer mouse, USB
 - Rinse cassettes (for maintenance of electrodes)
 - Power supply
 - Power cord



Warning! Do not substitute the power cord. A replacement cord with an incorrect rating can damage the instrument or power supply.

2.2 Installing the SageHLS

- 1. Open the LCD monitor box, and assemble it according to the manufacturer instructions
- 2. Using a box cutter, open the top seal on the SageHLS box, and open.
- 3. Remove the accessory box located just inside, atop the instrument. Remove the keyboard, mouse, and instrument power supply and cords from their packaging.
- 4. Firmly grip both sides of the **bottom** of the instrument and lift it along with the foam packaging inserts. The SageHLS weighs approximately **20 lbs**. Place the unit onto the bench top.



Warning! Do not lift the instrument by the lid or lid retainer. This can damage the mechanism or impede the function of the unit. Pressure should never be applied to the retainer, including sliding the instrument along a bench surface.



Lift from the bottom.



Do not lift by the retainer or lid.

5. Remove the foam insert which is on the inside of the lid.



Warning! After removing the instrument, open the lid and remove the foam insert and discard. The instrument can get damaged if operated with the insert in place.



- 6. Connect the LCD monitor (VGA port) to the SageHLS (VGA port, back panel) using supplied video cable. Connect monitor to the power outlet using the power supply supplied with monitor (**Figure 2.2**).
- 7. Insert USB connector from the computer **keyboard** into any USB port located on the back panel of the SageHLS (**Figure 2.2**).
- 8. Insert USB connector from the computer **mouse** into any USB port located on the back panel of the SageHLS (**Figure 2.2**).
- 9. Connect SageHLS instrument to the power outlet using the SageHLS power supply and power cable. The power input connector is in the lower left hand corner on the back panel of SageHLS, below the power switch (**Figure 2.2**).
- 10. Press power switch located on the rear of the instrument, and wait for software to launch (approximately 30 seconds)
- 11. When powered on, a <u>blue light</u> on the front panel of the Instrument will be on (Figure 2.3).

The SageHLS is ready for use. The software should automatically launch – allow 30 seconds.

Figures 2.2 and 2.3 show the rear and front panels of the SageHLS.







Figure 2.3. Front Panel of the SageHLS

After the power button is pressed, the front panel LED will light, and the software will launch.

2.3 Unpacking and Storage of Gel Cassettes

Store boxes in the upright position and confirm the following contents are present.

- 4 or 12 foil-sealed gel cassettes store at room temperature.
- 1 bag with reagent bottles
- 1 package of adhesive tape for sealing elution wells (in the reagent bag)

2.4 Unpacking Reagent Kits

SageHLS reagent kits can contain reagents with varying storage requirements. These will be noted on the with the documentation included with the kits and on the labels on the reagent bottles.



Warning! Storing reagents at the correct temperatures is required, refer to the documentation provided with reagent kits. Improper storage can affect system performance or reagent shelf-life. Contact support@sagescience for the replacement of reagents if necessary.

Introduction

Thank you for purchasing the SageHLS from Sage Science. We urge you to read this manual to familiarize yourself with the system's capabilities and precautions.



Warning! Use the SageHLS only as indicated in this operations manual. Injury or instrument damage can occur with improper usage.

The SageHLS is a platform on which users may purify intact DNA from cell suspensions and immobilize the DNA in the sample well as it enters the agarose gel surface. Once immobilized, the DNA can be enzymatically treated. The immobilized DNA is subsequently cleaved, electrophoretically size-selected, and collected in buffer in six wells.

3.1 System Overview

There are four components to the SageHLS system:

- Instrument The instrument is comprised of two heated gel cassette nests, an electrophoresis power supply, two electrode arrays embedded in the lid, and a single-board computer. The system computer is accessed by an external LCD monitor, mouse, and keyboard.
- **Software** System software allows the user to program run parameters to operate the system (e.g. heat, electrophoresis voltage, and time), operate the system (e.g. Start, Stop, Pause), and monitor operations (e.g. time remaining, electrophoresis current). The software also collects log files and allows users to review operation data from previous runs.
- **Gel Cassettes** Pre-cast, disposable agarose cassettes are manufactured by Sage Science. Gel cassettes have the capacity to process 2 samples per run.
- **Reagent Kits** -- Reagent kits are formulated by Sage Science and contain the necessary components to complete the processing of samples. This may include lysis reagents, conditioning buffers, enzyme mixes, electrophoresis buffers, or oligo adapters.

Heated nests for agarose gel cassettes (two samples per cassette).

Electrodes for electrophoresis are embedded in the lid.

The SageHLS instrument (the monitor and keyboard are not shown)

3.2 The Heated Nest

The SageHLS is equipped with a heated aluminum nest to control the reagent/enzyme reaction temperature conditions, and electrophoresis temperature. The nest accommodates the agarose gel cassette within which purification, enzymatic reactions, and electrophoresis occurs. The instrument is equipped with two nests, and each nest has two zones of temperature control:





Hot Surface! Use caution when using the SageHLS heated nest.

3.3 How the System Works

The SageHLS system uses pre-cast and disposable agarose gel cassettes. Each cassette has a 2-sample capacity.



A schematic of a single sample lane is shown below. It consists of an agarose column, with two wells into which reagents, buffers, or cell suspensions are added.

Electrophoretic current can be applied in two (perpendicular) directions. DNA is collected in six membrane-bound elution wells.



Single Sample Schematic

Cell suspensions are processed in three workflow stages:



STAGE 1. EXTRACTION

Cells are lysed. Electrophoresis is used to remove solubilized proteins and small nucleic acids. HMW DNA remains immobilized in the wall of the agarose sample well. •



DNA is subject to enzymatic treatment that cleaves the DNA into electrophoretically mobile fragment sizes. Cleavage may be random or targeted.



STAGE 3. COLLECTION

a. Electrophoresis (separation)

Electrophoresis separates DNA by size within the gel column.



b. Electrophoresis (elution)

Lateral electrophoresis elutes the DNA into 6 size-binned wells with buffer, and DNA is removed with wide-bore pipet tips.



3.4 Workflows

Cell Suspensions

Kits provided by Sage Science should be used to prepare cells, spheroplasts, or nuclei prior to DNA purification in the Sage HLS. <u>Cell suspension workflows may require steps</u> <u>one day before running the SageHLS.</u>

SageHLS

The processing of samples on the SageHLS requires user interactions with the instrument (opening and closing the lid), software (pausing and resuming), and reagent addition and removal from the cassette using a manual pipetter. <u>SageHLS workflows</u> <u>may require up to one day and an overnight run to complete</u>.

These interactions are guided by a **Workflow File**. A workflow file is comprised of three **Stages**, each of which consist of multiple **Steps**. Samples are processed by linking stages into a workflow.

Sage Science provides several pre-programmed workflow files for supported application. User can modify the pre-programmed workflows and save the customized workflows under new names.

Overview of sageHLS Workflow Stages

Stage 1: Extraction Stage - (cell suspension kit)

Extracts DNA from cell suspensions and removes residual byproducts.

Step types:

- Set sample well temperature.
- Electrophoresis voltage, time.
- Pause for operator action to add or remove sample and/or reagents.

Stage 2: Treatment Stage – (gel cassette kit)

Enzymatic treatment of extracte DNA (in sample well wall) with cleavase, targeted endonuclease, or other enzymatic processes

Step types:

- Set sample well temperature.
- Electrophoresis voltage, time.
- Pause for operator action to add or remove sample and/or reagents.
- Incubation time.

Stage 3: Collection Stage - (DNA size selection options)

Size Separation and collection (DNA size selection). These differ by target collection size requirements.

Step types:

- Separation electrophoresis
- Elution electrophoresis
- Reverse Elution electrophoresis

3.5 Workflow Summary

This workflow summary should be used as a guideline. Refer to specific Workflow Guides (Appendix A and B) for detailed workflow information.



Maintenance and Cleaning

4.1 Electrode Rinse: Weekly or After 5 Runs

Rinsing the SageHLS Electrodes is an important maintenance function. It is recommended that they be rinsed after every 5 runs, or at the weekly (whichever occurs first)

- 1. Place the blank SageHLS cassette bodies (provided by Sage Science) onto the Nests.
- 2. Completely fill the cassettes with deionized water.
- 3. Close the instrument Lid.
- 4. In the Main Tab, press the "Clear Run Data" button on the Commend Menu Bar.
- 5. In the cassette and protocol fields, enter the pre-programmed "Rinse Cassette" and "Rinse Protocol" options from the drop-down menus:
- 6. Press "Run" on the Command Menu. The protocol will take about 6 minutes to run.
- 7. Open the lid. Remove the rinse cassettes and pour out the wash into a drain.
- 8. Save the rinse cassettes for later use.

4.2 Cleaning the Nest: As Needed

Salts may accumulate on the nest from spilt buffer. The aluminum blocks should be cleaned, as needed, using deionized laboratory water. 70% ethanol may also be used, if users are concerned about biological cross contamination.

4.3 Warranty and Service

The SageHLS does not require field preventative maintenance other than what is described above. The system is subject to depot repair at the Sage Science facility. The warranty period is one year, and covers all expenses including parts, labor and shipping. The system is subject to full replacement at Sage Science's discretion.

SageHLS software

5.1 Workflow Editor Tab

In the SageHLS software, navigate to the **Workflow Editor Tab**. It is the second tab from the left:



The Stage List fields are populated with pre-set stage protocols.

5.2 Workflow File

A **Workflow File** links the three process **Stages**. The SageHLS instrument follows the Workflow file to run every **Step** of a process, in succession. The Workflow File uses a .shflow file extension.

Workflow Files are accessed by pressing the "Load Workflow" button in the Command Menu:

	Changes Pending			
New Workflow Load Workflow		undo Changes	Save Workflow	Save Workflow As
/				

Load workflow						×
$\leftrightarrow \rightarrow \cdot \uparrow$	Search Workflows		Q			
Organize 🔻 Nev	v folde	r				?
📌 Quick access	^	Name	Date modified 4/4/2017 3:33 PM	Type SHFLOW File	Size	1 KB
i OneDrive		HLS_CATCH 4-200kb.shflow	4/4/2017 3:33 PM	SHFLOW File		1 KB
This PC Desktop		HLS_CATCH 100-400kb.shtlow	4/4/2017 3:33 PM 4/4/2017 3:33 PM	SHFLOW File		1 KB 1 KB
 Documents Downloads 						
Music E Pictures						
Videos 🔛 OS (C:)						
💣 Network	~	<				>
	File na	me:	~	Workflow File (*.shflov OK	/) Cancel	×

A file list will pop-up from the SageHLS/Workflows folder.

When a Workflow File is selected;

a) the file name will appear in the Workflow File field,

	Workflow File	
9	HLS_HMW DNA Extraction	

b) the three Stage Protocols that comprise the workflow will become highlighted with a yellow background

Extraction Stage	Treatment Stage	Collection Stage
Extraction Stage	CATCH Method 1(HIT kit) Fragmentase Method 1(HEX kit)	Collection Stage
		V V

c) the Steps that comprise each highlighted Stage will appear in the Step Table. The step numbers include a prefix that corresponds to the Stage number. Each stage's step list ends with the step sub-number (i.e. 1-001 for Stage 1).

			Step Table
	~	Step	Description
Extraction	5	1-001	set temp: sample to 25.0°C, gel to 30.0°C; dwell time 00:02:00
Stage (1)	$\left \right $	1-002	pause for user action 'Replace sample well contents with 70ul of prepared sample. Replace reagent well contents with 230ul of
5 ()	X	1-003	separate for 01:00:00 at 55.0 V with wave index 1-1
		2-001	set temp: sample to 37.0°C, gel to 30.0°C; dwell time 00:10:00
Treatment	2	2-002	pause for user action 'Remove tape seal. Replace sample well contents with 80ul of reaction mix. Replace reagent well content
Stage (2)		2-003	incubate for 00:30:00
• • • •	U U	2-004	pause for user action 'Stop Reaction: Add 4ul 0.5M EDTA to sample well. Replace reagent well contents with 230ul of Lysis Buff
	0	3-001	set temp: sample to 25.0°C, gel to 'off'; dwell time 00:02:00
• • • •	J	3-002	separate for 00:45:00 at 55.0 V with wave index 3-1
Collection	\prec	3-003	separate for 01:15:00 at 55.0 V with wave index 3-3
Stage (3)		3-004	elute for 01:30:00 at 50.0 V with wave index 3-1
	- Ч	3-005	reverse for 00:00:05 at 25.0 V with wave index 3-1



Important! Stage Protocols may be edited or created in the Stage Editor. This is accessed in the factory setting using a super-user password. See Section 6.9.

The steps of any Stage Protocol can be viewed by highlighting the Stage Protocol name within a list.

A Stage Protocol can be de-selected by clicking on a blank field within the list. This will remove all of the Stage's steps from the Step Table.



The Workflow Editor screen can be reset by pressing the New Workflow button.



5.3 Saving a Workflow File

If a Workflow File has been modified by replacing or eliminating a step, it can be renamed and resaved using the "Save As" button.

		Changes Pending				
New Workflow	Load Workflow	0	-	Undo Changes	Save Workflow	Save Workflow As

The workflow file list will pop-up. Users may save new workflows or existing workflows with new file names.

Pre-set Workflows (with an HLS prefix), cannot be changed and re-saved. They may be saved with a new file name or modified and saved with a new file name.



5.4 Main Screen Tab

The Main Screen tab is the screen with which users interface with the SageHLS instrument. This includes initiating a run, monitoring a run, and pausing/resuming to conduct a manual pipetting step.

The default software screen on the SageHLS has a tabbed format. The Main Menu is the first tab.



5.5 Loading a Workflow File

1. If the System status in on Idle (there was a previous run), select "Clear Run" data to clear all fields and bring the system to an Idle System State:

Calibrate Detector	k Current 🛛 📫 R	Run Protocol	Force Elution	Stop Protocol	Clear Run Data	Display Info	Shutdown

2. Click the folder icon next to the Workflow File Field.



3. A Workflow File directory will open. Select a Workflow File and select "OK".

Load workflow						×
$\leftrightarrow \rightarrow \cdot \uparrow$	« Sa	Search Workflows		Q		
Organize 🔻 New	w folde	er				?
1 Orighteener	^	Name	Date modified	Туре	Size	
Y QUICK access		HLS_CATCH 1-15kb.shflow	4/4/2017 3:33 PM	SHFLOW File		1 KB
a OneDrive		HLS_CATCH 4-200kb.shflow	4/4/2017 3:33 PM	SHFLOW File		1 KB
This PC		HLS_CATCH 100-400kb.shflow	4/4/2017 3:33 PM	SHFLOW File		1 KB
Desktop		HLS_HMW DNA Extraction.shflow	4/4/2017 3:33 PM	SHFLOW File		1 KB
Documents						
👆 Downloads						
👌 Music						
Pictures						
🚰 Videos						
🛀 OS (C:)						
🔿 Network	~	<				>
	File na	ame:	~	Workflow File (*.shflow	N)	\sim
		L		ОК	Cancel	

4. The Workflow File name will populate the Workflow File field, the Workflow steps will populate the Step Table, and the System Status will indicate "ready"



- 5. Select the samples to be run by clicking the check box next to the sample position.
- 6. Enter Sample ID information into the Nest Configuration fields (optional). Sample IDs will be saved in the log file.



5.6 Temperature Test Workflow (Optional)

A preset workflow, named "Temp Test", is provided for users who wish to verify that the sample well temperature control is working properly. This test can be used for QC or IQ/OQ purposes. Note that the Temp Test workflow (run after the Current Test in the next section) requires approx. 10 minutes. After it is complete, a new workflow file must be loaded, and the Current Test re-run.

1. In the Workflow File field load the "Temp Test" workflow file.

_	Workflow File	
	Temp Test 🕒	

- 2. Run the Current Test (next section)
- 3. Run the "Temp Test" workflow (section 6.8).
- 4. At the end of the run, verify that the temperature profile in the Current and Temperature Monitor conforms to the Temp Test step values:



5.7 Running the Electrophoresis Current Test

Prior to loading a sample and running a cassette, the current test should be run to ensure that the electrophoretic properties of the cassette, based on measuring the electrical currents between electrodes, are within expected limits.

Before starting, make sure cassettes have been prepared (outlined in the Workflow Guides) and that they are placed on the nests that correspond to the sample locations that have been checked:



Important! Make sure that all cassette preparation steps in Section 5 have been completed.

The Current Test Procedure:

- 1. Close the SageHLS lid.
- 2. Once the lid is closed, press the "Check Current" button on the command button menu.

_							
Press							
"Check Curre	ent"						

3. A pop-up window will appear:

Ourrent C	heck				×
Cassette	01	02	-03	04	
Separation					
Elution					
	-	Start		Return	

4. Press "Start". The lid lock mechanism will engage, and the nest motor will activate. As the test progresses, a check mark will appear in the box corresponding to the electrophoretic path if the measured current falls within the expected limits.

Ourrent C	heck				×
Cassette	01	02	03	04	
Separation	\checkmark	\checkmark			
Elution	\checkmark	\checkmark			
	-	Start	×	Return	

5. If the test fails, the nest will automatically lower, and the lid will disengage until the issue is rectified.



Important! If a current check fails, check the buffer levels in the affected lane, and replace the buffer as needed. Contact Sage Science support if the current test continues to fail.

6. Press "Return" to return to the Main screen.

5.8 Running a Workflow

Press "Run Workflow".

Check Current	Run Workflow	Pause Workflow	Abort Workflow	×	Clear Run Data	0	Display Info	Shutdown
/								

The active step in the Workflow Step Table will be highlighted in yellow.

The System Status field will indicate the type of step being run.

Voltage, V		Lid Status	Lock Status	System Status				
0.0)			set temp				
		Step	Table					
Step	Step Description							
1-001	set temp: s	ample to 25.0°C, g	el to 30.0°C; dwell	time 00:02:00				
1-002	pause for u	ser action 'Replace	sample well conten	ts with 70ul of prep				
1-003	separate fo	r 01:00:00 at 55.0	V with wave index	1-1				
2-001	set temp: s	ample to 37.0°C, g	el to 30.0°C; dwell	time 00:10:00				
2-002	2-002 pause for user action 'Remove tape seal. Replace sample well conter							
2-003	2-003 incubate for 00:30:00							
2-004	pause for u	ser action 'Stop Re	action: Add 4ul 0.5	M EDTA to sample ι				
3-001	set temp: s	ample to 25.0°C, g	el to 'off'; dwell time	e 00:02:00				
3-002	separate fo	r 00:45:00 at 55.0	V with wave index	3-1				
3-003	separate fo	r 01:15:00 at 55.0	V with wave index	3-3				
3-004	elute for 01	:30:00 at 50.0 V w	ith wave index 3-1					
3-005	reverse for	00:00:05 at 25.0 \	/ with wave index 3	-1				

The "Time to User Event" timer will start a countdown until the next Pause step, which will require the lid to be opened, and reagent transfer steps with a pipette.



If a pause step contains instructions that exceed the Step Table display, hovering the mouse pointer over a step will display the complete instruction.

Voltag	e, V Lid Status Lock Status System Status		Electrophoresis Current and Sample W	ell
55.0	separate		80 - 75 -	
	Step Table		70-	
Step	Description		65-	
1-001	set temp: sample to 25.0°C, get to 30.0°C; dwell time 00:02:00		60 -	
1-002	pause for user action 'Replace sample well contents with 70ul of prec		55-	۲
1-003	separate for 01:00:00 at 55.0 V with wave index 1-1		50 -	
2-001	set temp: sample to 37.0°C, gel to 30.0°C; dwell time 00:10:00		¥ 45-	
2-002	pause for user action 'Remove tape seal. Replace sample well contents with	h 80ul of	of reaction mix. Replace reagent well contents with 230ul Enzyme Buffer (2C)'	
2-003	incubate for 00:30:00		e ar	
2-004	pause for user action 'Stop Reaction: Add 4ul 0.5M EDTA to		335-	
3-001	set temp: sample to 25.0°C, gel to 'off'; dwell time 00:02:00		30 -	
3-002	separate for 00:45:00 at 55.0 V with wave index 3-1		25-	÷
3-003	separate for 01:15:00 at 55.0 V with wave index 3-3		20 -	
3-004	elute for 01:30:00 at 50.0 V with wave index 3-1		15 -	
3-005	reverse for 00:00:05 at 25.0 V with wave index 3-1			
			10-	
			5-	Ī
				1
				:3
	,		Time, hh:mm	

When the instrument reaches a Pause step, a pop-up window will appear with instructions for a pipetting or manual procedure. When the manual procedure is complete, and the lid has been closed, users should press "OK" to continue the Workflow.



If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user action.

 Check Current		Run Workflow		Pause Workflow	Abort Workflow	Clear Run Data	Display Info	Shutdown
	Pa	use Wor	kflow					

After the last Pause step in the Workflow, the "Time to User Event" will begin to countdown to the end of the run, typically a few hours.

_	Time to User Event	
	03:29:59	

Throughout the workflow the "Electrophoresis Current and Sample Well Temperature" monitor will graphically display (in real time) the electrophoresis current for each sample, and the combined sample well temperature for each nest:



Sample Well Temperatures Nest 1 (Cassettes 1 and 2)

5.9 Other Run Commands

A workflow may be paused (and then resumed), or stopped at any time during a Workflow:

I-03

I-04

T-34



If a Workflow is manually paused, the following window will appear. Press "OK" to continue the Workflow.



If a Workflow is stopped, the following window will appear. Press "OK" to abort the protocol, or "Cancel" to return to the Workflow.

6	Warning	×
	Are you sure you want to abort the protocol?	^
	Cancel OK [Enter])

5.10 Running a Single Lane on a Cassette

A single lane can be run on a cassette allowing for the second lane to be used at a later date:

1. Make sure only the lane to be used is checked in the Nest Configuration in the Main Screen.

		Nest Configuration	
	Sample 1		
02	· · · · · · · · · · · · · · · · · · ·]

- 2. Save the original adhesive tape that the cassette was packaged with.
- 3. The unused lane can be left unsealed during the entire run 4. .
- 4. At the end of the run, re-seal the cassette with the original adhesive tape. Make sure to apply pressure (with a finger or lab marker) around all wells and chambers. With a marker, indicate which lane is unused.
- 5. Store the cassette at room temperature until needed.
- 6. The unused lane can be stored until the original expiration date of the cassette.

5.11 Stage Editor Tab

Workflow stages can be created or edited in the Stage Editor.

The Stage Editor screen is hidden under one level of user control, and can be accessed by entering a password:

1. In the Main Screen, press the "Display Info" button in the Command Menu.

<u>~</u>	Check Current	Run Protocol	Force Elution	Stop Protocol	×	Clear Run Data		Display Info	Shutdown
							1		
						/			

2. The "Display Info" window will appear. Press the "Advanced Tabs" button.



3. A Setup Password window will appear. Enter the password "pips":

Setup Password	



The Stage Editor will appear as a fifth tab on the right side of the screen.

5.12 Opening a Stage File

1. In the Command Menu, press "Load Stage":

		Changes Pending			
Load Stage	New Stage	<u> </u>	Undo Changes	Save Stage	Save Stage As

 A file folder window will appear listing the pre-set protocols in the "Preset Stages" folder. All stage files have a .shstage extension. Select a Stage file to load and press "OK"

🔿 🕐 🚺 « Sa	age Science > Sage HLS > Stages	S ~	Search Stages		2
rganize 🔻 🛛 New fold	ler			•	6
^	Name	Date modified	Туре	Size	
🖈 Quick access	CATCH 1-15kb.shstage	4/4/2017 3:33 PM	SHSTAGE File		6
OneDrive	CATCH 4-200kb.shstage	4/4/2017 3:33 PM	SHSTAGE File		6
This DC	CATCH 100-400kb.shstage	4/4/2017 3:33 PM	SHSTAGE File		6
	CATCH Method 1(HIT kit).shstage	4/4/2017 3:33 PM	SHSTAGE File		7
Desktop	Extraction Method 1.shstage	4/19/2017 4:12 PM	SHSTAGE File		6
Documents	Fragmentase Method 1(HEX kit).shstage	4/4/2017 3:33 PM	SHSTAGE File		6
Downloads	HMW Method 1.shstage	4/4/2017 3:33 PM	SHSTAGE File		6
Music					
Pictures					
Videos					
🛄 OS (C:)					
💼 lomega_HDD (F: 🗸	<				
Filer	name: Extraction Method 1.shstage	~	Stage Definition File	(*.shstage) `

3. When a Stage file is loaded, the following fields will populate:

	Stage File	
8	Extraction Method 1	

Stage File: The name of the loaded file

Stage Type	
extraction	

Stage Type: The type of stage assigned the loaded file (extraction, treatment, or collection)

Waveform Table								
Index	Α	В	С	D	Е	F	G	
1	0	0	0	0	0	0	0	1
2	150	50	30	10	3	1	81	1
3	300	100	30	10	30	10	45	
0								۲,

Waveform Table: Parameters for electrophoresis waveforms. Index 1 indicates direct current. Others are pulsed-field. This includes preset waveforms and any new waveforms that may have been programmed for the loaded protocol.

	Step Table	
Step	Description	
001	set temp: sample to 35.0°C, gel to 30.0°C; dwell time 00:02:00	
002	pause for user action 'Replace sample well contents with 70ul of prepared sample. Replace re-	
003	separate for 01:00:00 at 55.0 V with wave index 1	1
		1
		1

Step Table: The Step assigned to the loaded Stage file.

5.13 Changing the Stage Type

Click the arrows in the Stage type field to toggle between the three stages.

Stage Type	-	Stage Type
treatment		Collection
\sim		

5.14 Editing a Stage Step

A new Step can be programmed in the Stage Step fields:

	Stage St	ер		
Time, hh:mm:ss	Volts, V	Wave Index	Sample Temp, °C	Gel Temp, °C
00:00:00	0.0	1	20.0	(-) 30
	Prompt for Pau	ise State		
				Ĵ
	Time, hh:mm:ss	Time, hh:mm:ss Volts, V O0:00:00 O.0 Prompt for Paul	Stage Step Time, hh:mm:ss Volts, V Wave Index 00:00:00 0.0 1 Prompt for Pause State	Stage Step Time, hh:mm:ss Volts, V Wave Index Sample Temp, °C 00:00:00 0.0 1 20.0 Prompt for Pause State 20.0

There are several State/Actions that can be programmed. These are selected from a drop-down menu which appears when the "State/Action" field is clicked:

idle pause incubate	e tor 01:00:00 at 55.	u v with wave ind	iex 1		
separate elute reverse	Time, hh:mm:ss	Stage Str Volts, V	ep Wave Index	Sample Temp, °C	Gel Temp, °C
set temp	00:00:00	0.0	1	20.0	30

The programmable State/Action Steps are:

Pause: Pauses a run, and a pop-up window appears. User can enter instructions as text that will appear in the pop-up. Text is entered in the "Prompt for Pause State" field. No other parameters are entered.

		Stage St	-		
		stage st	ер		
State/Action	Time, hh:mm:ss	Volts, V	Wave Index	Sample Temp, °C	Gel Temp, °C
pause	00:00:00	0.0	1	20.0	× 30
		Prompt for Pau	use State		
					•

Set Temp: Sets the temperature of the sample well and gel column. The sample well can be set between 15-50°C. The gel column can be set to 30°C, or OFF. In a workflow, the temperatures will remain at the set points, until another "set temp" step is reached, or at the end of a run, when the heating function turns off.

		Stage Ste	ep		
State/Action	Time, hh:mm:ss	Volts, V	Wave Index	Sample Temp, °C	Gel Temp, °C
set temp	00:00:00	0.0	1	30.0	× 30
		Prompt for Pau	se State		
					*

Incubate: The instrument remains at an active state for a period of time, with no electrophoresis. This defines the amount of time that a temperature is applied to a sample. The amount of time is only parameter entered.

State/Action	Time, hh:mm:ss	Volts, V	Wave Index	Sample Temp, °C	Gel Temp, °C
incubate	00:00:00	0.0	1	30.0	30
		Prompt for Pau	ise State		
					~

Separate, Elute, and Reverse: Electrophoresis parameters. "Separate" is electrophoresis along the separation column. "Elution" is electrophoresis along the elution pathway (perpendicular to the separation pathway, into the elution wells). "Reverse" is the reverse direction of the Elution pathway - typically for a short reverse to release DNA from the elution well membrane. Time of electrophoresis, electrophoresis voltage, and Wave Index (a waveform from the Waveform Table) are entered.

pause		Stage Ste	p		
incubate ✓ separate elute reverse set temp	Time, hh:mm:ss	Volts, V	Wave Index 1 se State	Sample Temp, °C	Gel Temp, °C
					*
1					U .

5.15 Editing the Step Table

•

A step can be highlighted by clicking with within the step. Users can move the highlighted steps up and down the list by pressing the "Move Up" and "Move Down" buttons.

•

A highlighted step can be deleted by selecting the "Delete" button.

	Step Table		
Step	Description		1 Move Up
001	pause for user action 'Replace sample well contents with 70ul of prepared sample. Replace re-		
002	set temp: sample to 35.0°C, gel to 30.0°C; dwell time 00:02:00		Move Down
003	separate for 01:00:00 at 55.0 V with wave index 1		
		v	
1		_	Delete

A step can be added to the Step Table in several ways using the buttons on the left of the table:

Insert	Step			Descr	ription		
	001	pause f	for user action 'Replac	e sample well con	itents with 70ul of	prepared sample. Re	eplace rea
Append	002	set tem	p: sample to 35.0°C,	gel to 30.0°C; dv	vell time 00:02:00		
	003	separat	te for 01:00:00 at 55.	0 V with wave inc	dex 1		
Replace	004	separat	te for 02:00:00 at 75.	0 V with wave inc	dex 2		
Edit							V
	<u> </u>						
<u> </u>				Stage St	ep		
	State/Ac	tion	Time, hh:mm:ss	Stage St Volts, V	ep Wave Index	Sample Temp, °C	Gel Temp,
	State/Ac	tion	Time, hh:mm:ss	Stage St Volts, V	Wave Index	Sample Temp, °C	Gel Temp,
	State/Ac	tion	Time, hh:mm:ss	Stage St Volts, V 75.0	Wave Index	Sample Temp, °C	Gel Temp,
<u> </u>	State/Ac	tion	Time, hh:mm:ss	Stage St Volts, V 75.0 Prompt for Pau	Wave Index	Sample Temp, °C	Gel Temp,
<u> </u>	State/Ac	tion	Time, hh:mm:ss	Stage St Volts, V 75.0 Prompt for Pau	Wave Index 2 Use State	Sample Temp, °C	Gel Temp,

Insert: Inserts the programmed step in the table **<u>before</u>** the highlighted step.

Append: Appends the programmed step to the <u>end</u> of the list.

Replace: Replaces the parameters in the <u>highlighted</u> step.

Edit: Enters the parameters in the highlighted step into Stage Step fields.

5.16 Saving a Stage File

Press "Save Stage As" to save a Stage File. Preset protocols cannot be altered and saved with the same file name.

		Changes Pending			
Load Stage	New Stage		Undo Changes	Save Stage	Save Stage As
				(<u> </u>	
					7

After saving the new stage will appear in the Workflow Editor Screen.

CATCH Method 1(HIT kit)	
Fragmentase Method 1(HEX kit)	
New Stage	
	7

5.17 Log Files

At the end of every workflow, the SageHLS will save a log file (.txt) and an image file (.png) of the Main Screen at the end of the run. Additionally, a log file (.txt) is saved after every Check Current Test. The Table below summarizes the SageHLS log files.

File Type	Folder	Contents	Use
Workflow Log (.txt)	\Logs	Date/time, electrophoretic current data (mA), Temperature data, Sample ID, workflow stages and steps	View in Log Review screen; support for troubleshooting
End of Run screenshot (.png)	\Logs	Image of Main Screen at the end of the run	support for troubleshooting
Current Test Log	\QC	Date/time, electrophoretic current data (mA) from Current Test	support for troubleshooting

5.18 Log Review Tab

In the SageHLS software, navigate to the Log Review. It is the third tab from the left.



Sub-window navigation

The Log Review has four sub-windows:

Data Display	Stage Definition	Instrument Info	File Manager

- **Data Display (Default Window):** Shows Workflow information and collected data from a log file.
- **Stage Definition:** Shows parameter detail for each Stage in the workflow included from a log file
- **Instrument Info:** Display Instrument information (Name, serial number and software version) included in the log file.
- File Manager: A allows copying, deletion and transfer (to and from a USB storage device) of SageHLS files.

5.19 Log Data Display

From the Data Display sub-window, click the folder icon on the Log File field:

Log File		
P		Last Log
Workflow File		
	/	X
Opens folder files	s the /Logs to access	(Loads log file of the last workflow run on the system)

A window will appear listing the log files in the /Logs folder. Older log files are stored in subfolders that are automatically created and labelled by year/month (yyyy-mm).

Log files are automatically named with instrument name_serial number_date_ time (at end of run)_workflow name.

Select log file	AVAN VV MIT MIKUK IV KVIT VV KVI AV VI VV TRU TRUT KVM KAVUKUVIT K		×
← → × ↑ 📙 → This PC → OS (C:) → Users → Public → Public Docum	ments > Sage Science > Sage HLS > Logs > 2017-05 V 💍 Search 20	117-05	P
Organize 👻 New folder		BE 👻 🛄	8
 ✓ Inis PC ▲ Desktop 	Name SH_A123245_2017-05-02_17-02-31_HLS_HMW_DNA_Extraction	Date modified 5/2/2017 5:03 PM	1^ 1
> 🗄 Documents > 🖶 Downloads	SH_A123245_2017-05-02_17-03-36_HLS_CATCH_100-400kb SH_A123245_2017-05-04_15-30-36_HLS_CATCH_4-200kb SH_A123245_2017-05-05_09-32-47_HLS_HMW_DNA_Extraction	5/2/2017 5:31 PM 5/4/2017 3:30 PM 5/5/2017 9:32 AM	1
Fictures Wideos	 SH_A123245_2017-05-05_09-39-26_HLS_HMW_DNA_Extraction_3 SH_A123245_2017-05-05_10-11-27_HLS_HMW_DNA_Extraction_3 SH_A123245_2017-05-05_11-48-25_HLS_HMW_DNA_Extraction_3 	5/5/2017 9:58 AM 5/5/2017 11:47 AM 5/5/2017 11:55 AM	ר ו ו
> 🟪 OS (C:)	SH_A123245_2017-05-07_21-48-00_HLS_HMW_DNA_Extraction_3	5/8/2017 1:19 AM	1 ~ >
File name: SH_A123245_2017-05-02_17-03-36_HLS_CATCH_	100-400kb ~ Custom	Pattern (*.txt)	~
	ОК	Cancel	

When a log file is selected the data will populate the designated fields. The Data Display subwindow will display the Stage, Steps, Electrophoresis current and Sample well Temperature data.



The current and temperature are displayed as follows. The current (mA) is on the left y axis, and the temperature is on the right y-axis. Time (during the run) is on the x-axis.



5.20 Stage Definition Sub-Window

The Stage Definition sub-window displays the parameter details for each Step in each Stage for the Workflow that was associated with the loaded log file.



Stage Definition

The Stage Definition Window includes 2 slide bars. The lower slide bar scrolls between Stages. The upper slide bar scrolls between the Steps within the Stage.



5.21 Instrument Info Sub-Window

The Instrument sub-window displays instrument information that was associated with the loaded log file.

tain Screen	Workflow Editor	Log Review	System Options
	[Instrument Name Default Software Yesson 2.00 Premare Version 6.99 A122249	
	Lets Deploy	Stage Definition	Minager

Instrument Info

SageHLS Operations Manual Rev K 460034 6_1_2021

The Instrument Info displays information about the instrument and software revision of the system at the time the log file was collected.

Defa	ult
Software Version	Build Date
2.00	03-Apr-2017
Firmware Version	Serial Number
0.99	A123245

5.22 File Manager Sub-Window

The File Manager sub-window provides an interface for users to manage files on the SageHLS system, including Log files, Workflow files, and Stage files. The File manager allows the transfer of file between SageHLS system and an external USB drive.

Main Screen	Workflow Editor	Log Review	System Options	
S C:\Users\Public\Docume	HLS Folder Ints/Sage Science/Sage HLS/Debug	N	Flesh Folder	
	HLS Listing		Flash Listing	
○ (one level up)	System Files	E	xternal Storage device (USB drive)	
Go to Logs	Go to Workflows Go to Stages Go to	o QC Folder New Flash Folder	Copy to Flash Copy to HLS Eject Flash	
	👱 Dele	te Selected Refresh Listing		
	Data Display	age Definition Instrument Info	Fle Manager	

File Manager

Several buttons are associated with managing files on the SageHLS instrument, or between the instrument and an external USB drive.

Go to Logs Go to Workflows Go to Stages Go to QC Folder Image: Delete Selected Image: Delete Selected Image: Delete Selected					
Go to Logs Go to Workflows Go to Stages Go to QC Folder					
 Go to Logs: goes to the log file directory Go to Workflows: goes to the workflow file directory 					
 Go to Stages: goes to the stage file directory Go to QC folder: goes to the Current Test log file directory 					
Delete Selected: deletes files that are highlighted					
New Flash Folder Copy to Flash Copy to HLS Eject Flash Refresh Listing					

- New Flash Folder: creates a new folder on a connected USB device
- Copy to Flash: copies highlighted files from the SageHLS directory to a USB device
- Copy to HLS: copies highlighted files from a USB device to the SageHLS
- Eject Flash: allows safe removal of USB device
- Refresh Listing: deletes files that are highlighted

5.23 Copying Files To and From a USB Device

- 1. Insert a USB storage device into the USB port on the front panel of the SageHLS.
- 2. In the software, click on the Log Review Tab.

Main Screen	Workflow Editor	Log Review	System Options
		\	``````````````````````````````````````

3. At the bottom of the Log Review Screen, press the "File Manager" button.

Data Display	Stage Definition	Instrument Info	File Manager
		·	

4. Files located on the SageHLS system will appear in the directory window on the lefts side of the screen, and the files located on the USB device will appear on the right side of the screen.

	Main Screen		Workflow Editor	L	.og Review	System Options	1
Files stored on the SageHLS		Image: State State State State State State State State Image: State State State State State State State S	H.S.Polder er H.S.Lopp H.S.Lasting Lest.png test.but test.png -est.but -est.png -est.but -est.png () Co to Stages () Co ()	elete Selected Stage Definition	Fielder Fielder Image: State Stat	Copy to H.S Eject Flash	Files stored on the USB device

Buttons navigate to file folders containing types of SageHLS generated files.

5. Files can be highlighted by clicking the file names. Multiple files can be highlighted by holding the Shift button and clicking.

	Main Screen	Workflow Editor		Log Review	System Options
	B C: Users Public Doc	HLS Folder mentstjäge Science (Sage HLS Ludor HLS Ludoro			Rich Poder
Click to highlight. Shift-click to highlight multiple files.	(meteratus) 201743 201743 201744 201744 201744 201744 201745 201744 201745 20175	7-1205_1101-30_Hdes_test_prg 7-1205_1101-30_Hdes_test_prg 7-1205_10-3H-34_DC_Test_prg 7-1205_10-3H-34_DC_Test_prg 7-1212_1H-24-24_Useno-test_prg	Go to QC Folder Delete Selected Stage Definition		In Field Teach

6. Press "Copy to Flash" (or "Copy to HLS", if moving files from a USB Device to the SageHLS).

New Flash Folder	Copy to Flash	-	Copy to HLS	×	Eject Flash
Refresh Listing					

7. Press "Eject Flash" to safely unmount the USB device. The USB device can be removed from the USB port on the front panel.

5.24 System Options Tab

The System Options Tab is fourth Tab on the right. The time and date can be adjusted from this screen. The SageHLS may also be given an Instrument name ("Default" is the default name). These may be changed or edited. The "Changes Pending" light will flash if an edit is made. Press "Apply Changes" to accept the edits.



5.25 Updating SageHLS Software and Workflows

Extracting the file to a USB flash drive

SageHLS upgrade software is available for download at <u>www.sagescience.com/support/</u>. The files are provided in a zip file, and the contents must be extracted to the <u>root directory</u> of a USB flash drive:

- Download the Software Upgrade package by pressing the appropriate link on www.sagescience.com/product-support/sagehls-support/ (in the "Software Downloads" section).
- 2. Browsers may prompt users to save the file. The zip file will likely save to a "Downloads" folder on your computer. The file will be named [version number]-[instrument]-CD[cassette definition version].zip (e.g. v2.03-SageHLS-CD5.zip).



3. Open the zip file or unzip its contents onto your computer. It should contain a single folder with the same name as the zip file.

📕 🗹 📜 🖛	Compressed Folder Tools Downloads		💺 🗹 📜 🗧 Downloads	
File Home Share View	Extract		File Home Share View	
\leftarrow \rightarrow \checkmark \uparrow $ $ \Rightarrow This PC \Rightarrow Des	ktop > Downloads		\leftarrow \rightarrow \checkmark \uparrow 🐌 \diamond This PC \diamond Des	sktop > Downloads
Ouick access	▲ Name	\rightarrow	Ouick access	^ Name
	🦉 v2.03-SageHLS-CD5.zip			👃 v2.03-SageHLS-CD5
	Image: Image of the second	CD5 View		
	← → ↑ <mark>►</mark> Finis PC	, > Desktop	Name SageHLSUpdate	5

4. Within this folder is a subfolder called "SageHLSUpdate". Transfer this "SageHLSUpdate" folder into the <u>root</u> directory of a USB drive.



Upgrading SageHLS Instrument Software

- 1. Insert the USB drive with the upgrade folder into the USB port on the front panel of the SageHLS instrument.
- 2. From the Main Tab, Press "Display Info". This will open the information window.

Check Current 🗼 Run Workflow 👖 Pause Workflow 📕 Stop Workflow 🛛 Clear Run Data 🕐 Display Info			-						
	2	Check Current	-	Run Workflow	Pause Workflow	Stop Workflow	Clear Run Data	Display Info	Shutdown

3. From the information window, press "Update Software". This will open the Software Upgrade window (SageHLS Utility Applet). Press the "Update System" button.

Sage Science 500 Cummings Center, Suite 2400 Beverly, MA 01915 USA support@sagescience.com		sage	HLS [™]
supportesagesuchce.com		Sage HLS Utilit	y Applet v.1.6.0
sadeHI S™		Current Version	New Version
JUYCIILJ	\longrightarrow	03-Aug-2017	03-Aug-2017
Software Version: 2.03 Serial Number: HL00035 Firmware Version: 1.10 IP Address: 127.0.0.1 Build Date: 03-Aug-2017 MAC Address: 00-60-E0-68-B1-9D Update Software Advanced Tabs		Current Cassettes	New Cassettes
2 2017 Sage Science, http://www.sagescience.com Parts © 2017 Solidus Integration, http://solidusintegration.com		Update System Eject Flash Drive	Shutdown Start Sage HLS
Parts © 2017 National Instruments, http://ni.com		1	
Update Software	Upo	late System	

4. A warning window will appear. Press "OK":

() Warning	×
Original files with the same names will be overwritten!	
Cancel OK [Enter]	

5. After the files have copied, a second window will appear. Press "OK".

🕚 Warning		×
	23 files copied; 0 files deleted	×
	OK [Enter]	

6. Press "Eject Flash Drive", then remove the USB drive.

Update System Eject Flash Drive	Shutdown Start Sage HLS
---------------------------------	-------------------------

7. Press "Start SageHLS" to return to the main screen. The upgrade will be installed and the instrument ready to use.



8. To check that the software instrument has been updated, press "Display Info" again. In the information window, the software version number is listed on the left side:



Getting Started

Workflow guides for preparing HLS-compatible cell suspensions are separately with purchases of Cell Preparation Kits. That may also be viewed or downloaded at http://www.sagescience.com/product-support/sagehls-support/

- Mammalian White Blood Cell from Whole Blood Preparation
- Mammalian Tissue Culture Cell Preparation
- E.Coli Sphroplast Perparation

Application workflow guides are individually provided with the purchase of Gel Cassette Kits and may also be viewed or downloaded from http://www.sagescience.com/product-support/sagehls-support. These include complete step-by-step instructions for SageHLS workflows and associated instructions.

- HLS-CATCH Targeted Collection of HMW Genomic Regions
 - Sourcing guide for for Guide-RNAs and qPCR Primers: HLS-CATCH BRCA1 Enrichment in Human or Mouse
 - Cas9 guide-RNA Design Tutorial for HLS-CATCH
- High Molecular Weight DNA Extraction

Before starting, users should be aware of the following and prepare accordingly:

- Some kits may require the purchase of reagents from 3rd party suppliers
- Some cell suspensions may require preparation steps one or more days before a SageHLS run
- Some SageHLS runs require an overnight electrophoresis step



Important! New workflows are under development, and existing workflows may be subject to improvements or modifications. Make sure to check the Revision and Release Date of this document with the Manual or Guides posted on the SageHLS support page on the Sage Science website (<u>http://www.sagescience.com/product-support/sagehls-support</u>/).

S sage science